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Lab Resource: Stem Cell Line

Derivation of Genea042 human embryonic stem cell line

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ABSTRACT

The Genea042 human embryonic stem cell line was derived from a donated, fully commercially consented ART blastocyst, through ICM outgrowth on inactivated human feeders. The line showed pluripotent cell morphology and genomic analysis verified a 46, XX karyotype and female allele pattern through traditional karyotyping, CGH and STR analysis. Pluripotency of Genea042 was demonstrated with 81% of cells expressing Nanog, 95% Oct4, 53% Tra1-60 and 97% SSEA4, a PluriTest Pluripotency score of 30.06, Novelty score of 1.24 and Alkaline Phosphatase activity. The cell line was negative for Mycoplasma and any visible contamination.

Resource table

Name of stem cell line	Genea042 (Alternate ID: SIVF042)
Institution	Genea Biocells
Person who created resource	Biljana Dumevska
Contact person and email	biljana.dumevska@geneabiocells.com
Date archived/stock date	March, 2009
Origin	Human Embryos
Type of resource	Derived human embryonic stem cell line
Sub-type	Human pluripotent cell line
Key marker expression	Nanog, Oct4, Tra1-60, and SSEA4
Authentication	Identity and purity of cell line confirmed (Figs. 1–6 below)
Link to related literature (direct URL links and full references)	(Laurent et al., 2011) http://www.ncbi.nlm.nih.gov/pubmed/?term=21211785
Information in public databases	National Institutes of Health (NIH) registered *NIHhESC-13-0231* UK Stem Cell Bank (UKSCB) registered SCSC14-40 SNP Data GEO accession numbers GSM638444
Ethical Approval	Obtained from the Genea Ethics Committee on 21 February 2001 under the Australian National Health and Medical Research Council (NHMRC) licence 309,703

Resource details

Date of derivation	February 2009
Karyotype	46, XX – no abnormalities detected
Sex	Female
Pluripotent	YES - by Alkaline Phosphatase stain positivity, Nanog, Oct4, Tra1-60, and SSEA4 staining and PluriTest
Disease status	Unaffected
Sterility	The cell line is tested and found negative for Mycoplasma and any visible contamination
Sibling lines available	YES - GENEA043 (XY NIHhESC-13-0232, SCSC14-40)

Materials and methods

Cell line derivation

The embryo was plated whole onto mitomycin C inactivated Detroit 510 HFF human feeders (plated 90,000 cells 1 well of 4 well – 47,368 cells/cm^2) in 20% Knock out serum in standard hESC culture medium with 20 ng/mL Fgf2 (Amit et al., 2000) and 100 ng/mL GMCSF (R&D Systems #215-GM). CGH karyotyping and STR profiling was performed at the first cryobanking step from ICM outgrowths maintained on feeders. Alkaline Phosphatase staining was performed on feeders. Cells were then enzymatically passaged as single cells in M2 pluripotent cell maintenance medium (Genea Biocells) and CGH/karyotyping repeated, immunofluorescent pluripotent marker staining, PluriTest and sterility testing performed.

Genetic analysis

1. *Karyotyping*: Passage 4; For enrichment of metaphase cells, cellular outgrowths were incubated with either 0.22 ng/ml colcemid (Invitrogen) and 37.5 g/ml BrdU (Sigma) for 17–19 h or 5 ng/ml colcemid for 2.5 h. Single cells were subsequently obtained using Non-Enzymatic Cell Dissociation Solution (Sigma) and transferred to Sydney IVF's (now Genea) diagnostic cytogenetics laboratory for preparation of metaphase spreads and cytogenetic analysis by G-banding. A minimum of 15 metaphase cells were examined, of which a full karyotype or chromosome counts was performed for 5 and 10 of the metaphases respectively.

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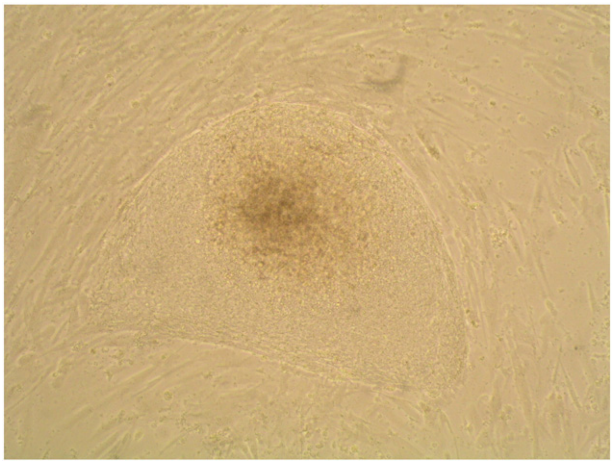

Fig. 1. Brightfield morphology of Genea042 on human inactivated feeders.

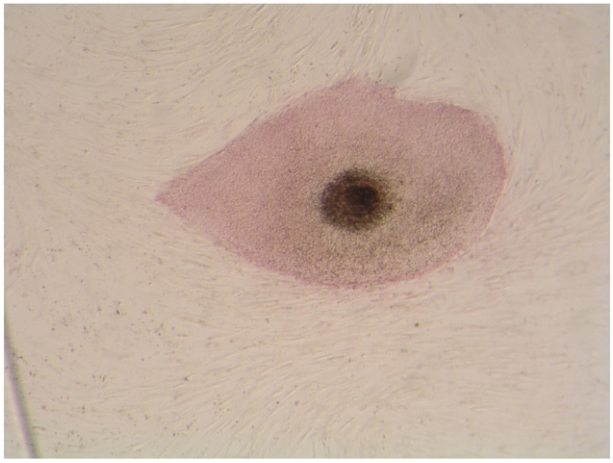

Fig. 2. Alkaline Phosphatase staining.

Table 1
CGH array summary.

Parameter	Result
Sample name	Genea042 p8_2
Date reported	7th August 2013
Hybridisation balance	A balanced hybridization was observed for all chromosomes, relative to reference DNA
Copy number change	No copy number changes >400 kb were detected
Interpretation	Female cell line

2. *Comparative Genomic Hybridisation (CGH) based chromosomal analysis:* Passage 8 (6 on feeders, 2 enzymatic); CGH was used to screen targeted regions of the genome for gains and losses associated with chromosomal imbalances such as aneuploidy, deletions and duplications. CGH was performed using SurePrint G3 microarrays (8 × 60 K format) which were scanned with the Agilent Scanner C and analysed using Genomic Workbench Standard Edition 5.0 software (Agilent Technologies).
3. *DNA Profiling:* Passage 3; DNA 'fingerprinting' was performed using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems #4,322,288) to provide permanent genetic identification of the cell lines. https://www.thermofisher.com/order/catalog/product/4322288.

Pluripotency assessment

1. *Alkaline Phosphatase*: Passage p4; Genea042 grown on feeders were stained as per manufacturers protocol using the Merck Millipore Alkaline Phosphatase Detection Kit (SCR004)
2. *Immunofluorescence*: Passage 17 (15 on feeders, 2 enzymatic); cells were fixed with formalin and stained with Nanog #560,483 1:200; Oct4 #560,217 1:150: Tra1-60 #560,121 1:150; SSEA4 #560,308 1:200 (all BD Pharmingen:). Images were acquired with an IN Cell Analyser 6000 and quantified using In Cell Developer Software (GE).

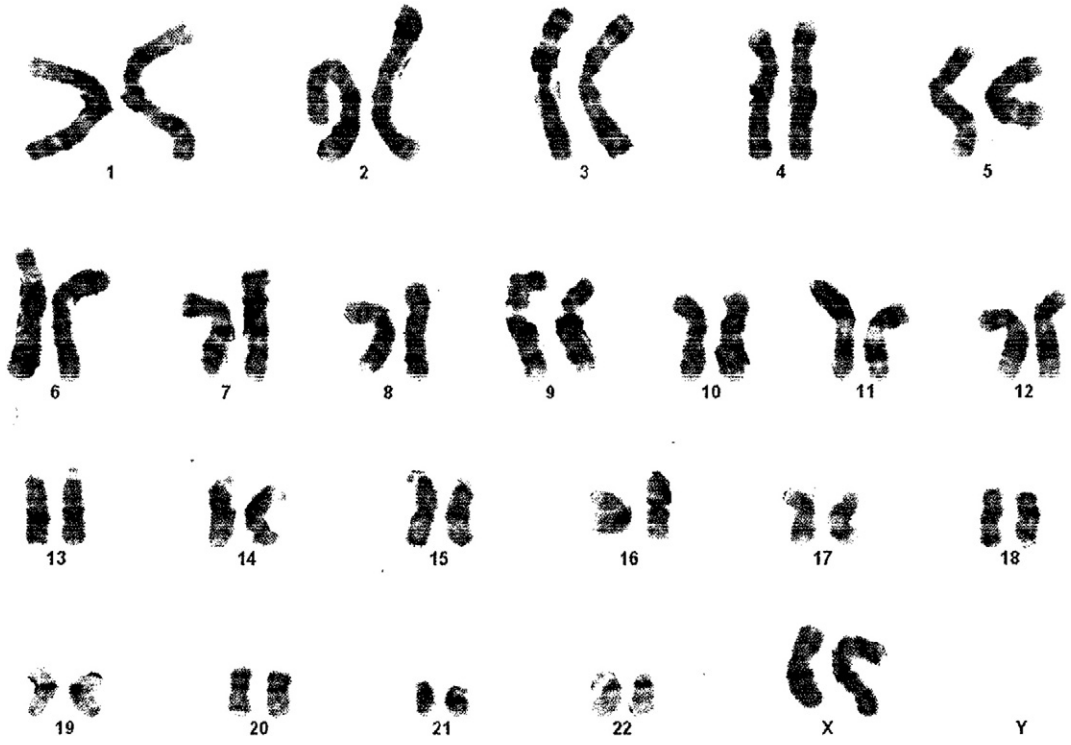

Fig. 3. Karyotype.